PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P867PC00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00935	International filing date *(day/month/year)* 23.12.2003	Priority date *(day/month/year)* 23.12.2002
International Patent Classification (IPC) or both national classification and IPC G01N33/497		
Applicant UNISENSE A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.07.2004	12.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Komenda, P Telephone No. +49 89 2399-2777

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00935

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-64 as originally filed

Claims, Numbers

1-70 filed with telefax on 14.03.2005

Drawings, Sheets

1/20-20/20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 60,61

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 60,61

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-47,49-59,62-67,69
	No:	Claims	48,68,70
Inventive step (IS)	Yes:	Claims	1-47,49-59,64-67
	No:	Claims	48,62,63,68-70
Industrial applicability (IA)	Yes:	Claims	1-59,62-70
	No:	Claims	1-59,62-70

2. Citations and explanations

see separate sheet

Section IV:

There are multiple (groups of) inventions in this international application:

1. Claims: 1-47, 49-59, 62-70

   Claims 1-47, 49-59 and 62-70 directed to a device and a method for non-invasive measurement of an individual metabolic rate

2. Claim 48

   Claim 48 directed to a method for regulating metabolite supply to a metabolising particle during culturing

Section V:

Reference is made to the following documents:

D1: EP-A-1 134 583
D2: EP-A-0 448 923
D3: SHIKU H ET AL: "Oxygen consumption of single bovine embryos probed by scanning electrochemical microscopy." ANALYTICAL CHEMISTRY. UNITED STATES 1 AUG 2001, vol. 73, no. 15, 1 August 2001 (2001-08-01), pages 3751-3758, ISSN: 0003-2700

N: Document D1 describes a method and device for non-invasive measurement of the biological activity of a substantially spherical metabolising particle, wherein the device comprises a compartment, capable of comprising a medium and at least one detector for measuring the concentration of a metabolite inside the compartment (see paragraphs 0022-0026).
The device of independent claim 1 additionally recites, besides the above features, that "said compartment is defined by a diffusion barrier arranged around the said spherical metabolising particle to restrict and reduce the diffusive flux of metabolites to and from the particle and allowing metabolite transport through the diffusion

barrier". A similar distinguishing feature is also present in method claims 36 and 64 (Article 33(2) PCT).

Independent claims 48, 68 and 70 are silent with respect of the above distinguishing feature(s) and are thus anticipated by document D1 (see paragraphs 0022-0024). Only claims 49 to 59 include again said distinguishing feature(s).

IS: By placing the metabolising particle in a compartment which limits the diffusive supply/removal of metabolites, changes in the concentration inside the compartment of these metabolites can be better detected and the method becomes more accurate. The problem to be solved is thus to provide a device/method for accurate non-invasive measurement of biological activity of metabolising particles.
Documents D2 and D3 also relate to detection of biological activities in a metabolising specimen. However, they do not deal with determinations of metabolising rate and establishing diffusion gradients by means of a diffusion barrier and thus do not disclose a solution for the above problem. Thus an inventive activity is acknowledged for claims 1-47, claims 49 to 59 and claims 64-67 (Article 33(3) PCT).

IA: For the assessment of the present claims 1-70 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the uses of human embryos for industrial or commercial purposes (which is intended with the present application).

PCT/DK03/00935
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P 867 PC00

Claims amended in response to the First Written Opinion

1. A device for non-invasive measurement of the individual metabolic rate of a substantially spherical metabolizing particle, which device comprises

a) at least one compartment, said compartment being defined by a diffusion barrier and capable of comprising a medium with a substantially spherical metabolizing particle, said diffusion barrier is arranged around the substantially spherical metabolizing particle to restrict and reduce the diffusive flux of metabolites to and from the particle, allowing metabolite transport through the diffusion barrier to and/or from the substantially spherical metabolizing particle by means of diffusion whereby a metabolite diffusion gradient is allowed to be established from the substantially spherical metabolizing particle and throughout the medium,

b) at least one detector for measuring the concentration of a metabolite inside the compartment.

2. The device according to claim 1, wherein the diffusion barrier is constituted by a compartment wall having at least one metabolite permeable opening and the medium.

3. The device according to claim 2, wherein the compartment wall is produced from a substantially metabolite impermeable material.

4. The device according to according to claim 3, wherein the substantially metabolite impermeable material has a metabolite diffusion coefficient less than 1 % of the metabolite diffusion coefficient in water, particularly less than 0.2%, most particularly less than 0.05%.

5. The device according to any of the claims 2-4, wherein the metabolite flux through the compartment wall of substantially metabolite impermeable material constitutes less than 10 % of the total metabolite flux to the compartment, particularly less than 1%, most particularly less than 0.1%

6. The device according to claim 3, wherein the substantially gas impermeable material is selected from the group of materials of plastics, polymer material, glass material, metallic material, and ceramic material as well as combinations thereof.

7. The device according to claim 6, wherein the polymer material is selected from the group of polymers of acetal resins, acrylic resins, cellulosic plastics, fluoroplastics, ionomers, parylenes, polamides, polyamide nanocomposites, polycarbonates, polyesters, polyimide, polyolefins, polyphenyle sulfides, polysulfones, styrenic resins,vinyl resins, plastic alloys, multiplayer polymers, epoxy resins, olefins thermoplastic elastomers, polyether block amides, polybutadiene thermoplastic elastomers styrenic thermoplastic elastomers, vinyl thermoplastic elastomers, rubber materials such as butadiene rubber, butyl rubber, bromobutyl rubber, chlorobutyl rubber, polyisobutylene rubber, chlorosulfonated poluethylene rubber, epichlorohydrin rubber, ethylene-propylene rubber, fluoroelastomers, natural rubbers, neoprene rubbers, nitrile rubbers, polysulfide rubbers, polyurethane rubbers, silicone rubbers, styrene-butadiene rubbers or copolymers thereof.

8. The device according to claim 1, wherein the diffusion barrier is constituted by a high-viscosity medium.

9. The device according to claim 8, wherein the high-viscosity medium is due to a high concentration of organic solutes selected from the group of dextrans, glycerol, sugars, carbohydrates, proteins, and inorganic salts.

10. The device according to any of the preceding claims, wherein the shape of the compartment is selected from the group of a cylinder, a polyhedron, a cone, a hemisphere or a combination thereof.

11. The device according to claim 10, wherein the general shape of the compartment is a cylinder.

12. The device according to any of the preceding claims comprising an insert for the adjustment of the transverse dimension of the compartment.

13. The device according to any of the preceding claims, wherein the compartment has an adjustable bottom in order to change the dimensions and either increase or decrease the compartment volume.

14. The device according to any of the preceding claims, wherein the transverse dimension is less than 2.5 mm, particularly less than 1.5 mm, more particularly less than 500 µm, such as less than 250 µm.

15. The device according to claim 12, wherein the transverse dimension of the insert is less than 1.5 mm, particularly less than 1.0 mm, more particularly less than 500 µm, even more particularly less than 300 µm.

16. The device according to any of the preceding claims wherein the longitudinal dimension of the compartment is between 2 mm to 25 mm, particularly between 3 mm to 15 mm.

17. The device according to claim 2, wherein the metabolite permeable opening is constituted by a metabolite permeable membrane.

18. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising silicone, Teflon fluoropolymers, or plastic compounds such as polyethylene, polypropylene or neoprene.

19. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising permeable matrixes or porous material such as glass, ceramics, minerals, glass or mineral fibers, or precious metal such as gold or platinum.

20. The device according to claim 17, wherein the metabolite permeable membrane is produced from a material comprising silicone.

21. The device according to any of the preceding claims, wherein a metabolite permeable layer is arranged in the bottom of the at least one compartment.

22. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising silicone, Teflon fluoropolymers, plastic compounds such as polyethylene, polypropylene or neoprene.

23. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising permeable matrixes or porous material such as glass, ceramics, minerals, glass or mineral fibers, or precious metal such as gold or platinum.

24. The device according to claim 21, wherein the metabolite permeable layer is produced from a material comprising silicone.

25. The device according to any of the preceding claims 21-24 , wherein the thickness of the metabolite permeable layer is at least 100 µm, particularly at least 300 µm, and more particularly at least 900 µm.

26. The method according to any of the preceding claims, wherein the metabolite detector is placed at the bottom of the compartment.

27. The method according to any of the claims 21-26, wherein a metabolite permeable layer is placed between the substantially spherical metabolizing particle and the metabolite detector.

28. The method according to any of the claims 21-27, wherein the metabolite permeable layer has a thickness of at least twice the diameter of the substantially spherical metabolizing particle.

29. The device according to any of the preceding claims, wherein the metabolite is a gas.

30. The device according to any of the preceding claims, wherein the metabolite is oxygen or carbon dioxide.

31. The device according to any of the preceding claims, wherein the detector is an oxygen detector.

32. The device according to claim 31, wherein the detector for measuring the oxygen concentration comprises amperometric oxygen sensors, membrane inlet mass spectrometry, microspectrophotometry, or optical oxygen sensing.

33. The device according to claim 32, wherein the optical oxygen sensing is performed using a luminophore, particularly an immobilized luminophore placed inside the compartment, more particularly in the bottom, and a detector of luminescence.

34. The device according to claim 33, wherein the luminophore comprises Ruthenium(II)-tris-4,7-diphenyl-1,10-phenatroline per chlorate (Rudpp) immobilised in a polystyrene matrix, Ruthenium (II) tris-1,7-diphenyl-1,10-phenanthroline chloride, Ruthenium(II)-tris(bipyridyl) complex, Tris (2,2'-bipyridyl di-chloro-ruthenium) hexahydrate, Ru(bpy), Platinum (II)-octa-ethyl-porphyrin in polystyrene, Platinum (II)-octa-ethyl-porphyrin in poly(methyl-methacrylate), Platinum (II)-octa-ethyl-keto-porphyrin in polystyrene, Platinum (II)-octa-ethyl-keto-porphyrin, Palladium (II)-octa-ethyl-porphyrin in polystyrene, Platinum-1,2-ene-dithiolates class of compounds.

35. The device according to claim 33, wherein the detector of luminescence is a luminescence reader, a photomultiplier tube or a CCD camera (12).

36. A non-invasive method for determining the metabolic rate of a substantially spherical metabolizing particle, comprising

a) providing at least one device as defined in any of claims 1-35,

b) arranging a substantially spherical metabolizing particle in the medium of a compartment,

c) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure, and

d) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle.

37. The method according to claim 36, wherein metabolite is supplied to the substantially spherical metabolizing particle by diffusion through the medium.

38. The method according to any of the claims 36-37, wherein the substantially spherical metabolizing particle is cultured in the compartment.

39. The method according to any of the claims 36-38, wherein the metabolite concentration is measured in a volume smaller than the volume of the compartment and/or the volume of the medium.

40. The method according to any of the claims 36-39, wherein the metabolic rate of said substantially spherical metabolizing particle is determined by determining a metabolite diffusion gradient in the compartment based on the measured metabolite concentration, and correlating said metabolite diffusion gradient to the metabolic rate of said substantially spherical metabolizing particle.

41. The method according to any of the claims 36-40, wherein at least two measurements of the metabolite concentration are performed.

42. The method according to any of the claims 36-41, wherein the metabolite concentration is a gas partial pressure

43. The method according to claim 42, wherein the gas partial pressure is the partial pressure of oxygen or carbon dioxide.

44. The method according to any of the claims 36-43, wherein gas is supplied to the substantially spherical metabolizing particle by diffusion through the stagnant medium in the compartment directly from the atmosphere or from a larger volume of medium in equilibrium with the atmosphere.

45. The method according to any of the claims 36-44, wherein the substantially spherical metabolizing particle is selected from the group of an embryo, group of cells, such as cancer cell(s), stem cells, embryonal stem cells, C. elegans or other small multicellular organisms.

46. The method according to claim 45, wherein the substantially spherical metabolizing particle is an embryo.

47. The method according to any of the preceding claims 36-46, wherein the measurement of the concentration of the metabolite is conducted after a temporary elimination of diffusive metabolite supply to the compartment from outside the compartment.

48. A method for regulating metabolite supply to a substantially spherical metabolizing particle during culturing, comprising

a) providing at least one device comprising a compartment with a medium,

b) culturing a substantially spherical metabolizing particle in the medium of a compartment,

c) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure, and optionally

d) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle and optionally

e) e) regulating the metabolite supply depending on the metabolite concentration measure and/or the metabolic rate of said substantially spherical metabolizing particle.

49. The method according to claim 48, wherein at least one of the devices is as defined in any of claims 1-35,

50. The method according to claim 48 or 49, wherein the metabolite is a gas.

51. The method according to claim 50, wherein the metabolite is oxygen and the metabolic process is respiration.

52. The method according to claim 48 or 49, wherein the regulation is conducted by changing the metabolite concentration outside the compartment.

53. The method according to claim 48 or 49, wherein the regulation is conducted by changing the dimensions of the compartment.

54. The method according to claim 53, wherein the volume is adjusted by inserting an insert.

55. The method according to claim 53, wherein the transverse dimensions of the compartment is adjusted by inserting an insert.

56. The method according to claim 53, wherein the volume is adjusted by shifting the position of an adjustable bottom of the compartment.

57. The method according to claim 53, wherein the regulation is conducted by changing the diffusion barrier of the compartment.

58. The method according to claim 53, wherein the diffusion barrier is changed by changing the thickness of a compartment wall.

59. The method according to claim 53, wherein the regulation is conducted by changing the size of at least one opening in the compartment wall.

60. A method for selecting a viable embryo comprising,

a) determining the metabolic rate of the embryo at least once during culturing , and

b) selecting the embryo having an optimal metabolic rate.

61. The method according to claim 60, wherein the determination of the metabolic rate is conducted without causing any change in the growth conditions experienced by the embryo.

62. The method according any of the claims 60-61, wherein the metabolic rate is measured in a device as defined by any of the claims 1-35.

63. The method according any of the claims 60-61, wherein the metabolic rate is determined by a method as defined in any of claims 36-47.

64. A non-invasive method for determining the metabolic rate of a metabolizing particle, comprising

a) providing at least one device as defined in any of claims 1-35,

b) culturing a metabolizing particle in the medium of a compartment,

c) reducing metabolite supply to the medium during at least a part of the culturing period,

d) measuring a metabolite concentration inside the compartment obtaining a metabolite concentration measure after the metabolite supply has been reduced, and

e) correlating said metabolite concentration measure to a metabolic rate of said substantially spherical metabolizing particle.

65. The method according to claim 64, wherein the metabolite is oxygen and the metabolic rate is the respiration rate.

66. The method according to claim 64, wherein the oxygen supply is reduced to zero.

67. The method according to claim 64, wherein the gas partial pressure measure in the compartment has been obtained during the period of reduced oxygen supply

68. A culture device for culturing a metabolizing particle, which device comprises at least one compartment, said compartment being defined by a diffusion barrier and capable of comprising a medium with a metabolizing particle, said diffusion barrier allowing metabolite transport to and/or from the metabolizing particle by means of diffusion, whereby a metabolite diffusion gradient is allowed to be established from the metabolizing particle and throughout the medium.

69. The device according to claim 68, wherein said device has one or more of the features as defined in any of claims 1-35.

70. A method for culturing a metabolizing particle, said method comprising

a) providing at least one device as defined in any of claims 68-69,

b) arranging a metabolizing particle in the medium of the compartment, and

c) culturing the metabolizing particle.